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Role of Fe-oxidizing bacteria in metal bio-corrosion in the marine environment

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**14. ABSTRACT**
This report summarizes investigations on the role that lithotrophic iron-oxidizing in biocorrosion of mild steel. The first finding is that obligate iron-oxidizers belonging to the Zetaproteobacteria can readily grow on mild steel as an Fe(II) source, and that steel coupons incubated in natural seawater are rapidly colonized by these bacteria. This is the first demonstration these bacteria exist outside of marine hydrothermal vents. These bacteria are early colonizers of steel surfaces and through their formation of a thick biofilm may encourage other members of bio-corroding community to develop. New isolates that resulted from this work are informing us about the physiology and genetics of this novel group of bacteria.

**15. SUBJECT TERMS**
Microbially influenced corrosion (MIC), iron, iron-oxidizing bacteria, mild steel, lithotrophs, Mariprofundus, biocorrosion

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Cover Page:
**GRANT #** : #N00014-08-1-0334
**PRINCIPAL INVESTIGATOR** : Dr. David Emerson
**INSTITUTION :** Bigelow Laboratory for Ocean Sciences
**GRANT TITLE :** Role of Fe-oxidizing bacteria in metal bio-corrosion in the marine environment.

**AWARD PERIOD :** January 1, 2008 – March 31, 2015

**OBJECTIVE :** The primary objective of this project was to understand the role that the iron-oxidizing bacteria, a unique group of chemolithoautotrophic microbes, play in the corrosion of steel. Despite significant research showing that microbially-induced corrosion (MIC) is an important contributor to the corrosion and destruction of steel structures, almost no previous work has been done to show what effects Fe-oxidizing bacteria may have on this process. Corrosion is a major issue for the Naval fleet, as well as fixed and floating assets that support fleet operations. Four lines of inquiry were identified in line with the primary objective.

- To understand the extent to which FeOB are present in active steel bio-corrosion, and the role that they may play in the process.
- To document the presence of FeOB in natural and artificial corrosion sites and quantify their abundance, and diversity of relative to other known microbial players in corrosion such as FeRB.
- Understand potential synergistic interactions between FeOB and other bacteria in the corrosion process.
- Develop a better understanding of the physiology of Fe-oxidation using comparative approaches, and laboratory microcosms.

## APPROACH:

- *Field Studies*

1. Exposure experiments - Expose mild steels in open seawater and at sediment water interfaces to determine the level of corrosion and the extent to which FeOB are involved. Microbial communities were analyzed using a variety of culture-independent and cultivation-based approaches. These include fluorescent in situ hybridization (FISH) using primers specific for groups of FeOB, quantitative PCR (qPCR) using primers specific for FeOB and specific groups of sulfate-reducing bacteria and methanogens, as well as cultivation based most probable number (MPN) analysis for Fe-reducing bacteria. We will use tagged pyrosequencing as a tool to investigate the microbial communities that develop.

2. Singe cell genomic analysis of natural corrosion communities. We utilized the single cell genomics center at Bigelow to isolate single cells (single amplified genomes (SAGs) from corrosion products and identify these cells by 16S rRNA gene sequencing. SAGs of interest, i.e. belonging to the Zetaproteobacteria, or otherwise

dominant members of the corrosion community, will be selected for whole genome sequencing.

3. Surface corrosion analysis - Some of this work was done in conjunction with Dr. Little's group at NRL using environmental scanning electron microscopy coupled with an energy dispersive X-ray spectrophotometry, as well as profilometry. Recent analysis of surface attachment, colonization, and growth experiments have been done at Bigelow.

4. A microcosm system was developed to study attachment and growth of Fe-oxidizing bacteria in controlled conditions in the laboratory and then image the biofilms to understand what impact they have on the process.

## ACCOMPLISHMENTS:

The primary accomplishments for the project are listed as bullets in this section.

• Completed studies tracking the colonization of microbial communities on steel coupons that are exposed to natural seawater at two different locations, an estuarine environment, and a small bay with full strength seawater. Assessment of the communities suggests there are some similarity in communities; however there was a noticeable difference in trends in the population dynamics. In the estuarine environment there was an initial increase in the abundance of Zetaproteobacteria, followed by a decrease in their numbers, while numbers of qPCR of dissimilaratory sulfate reductase genes (DSR) genes, increased with time and were stable during later sampling periods. In the marine sample, the abundance of SRB was greater at an earlier time and remained quite stable, while the Zetaproteobacteria did not become more prevalent until later in the colonization period. There were noticeable differences between the estuarine and seawater communities, with different patterns of colonization. Betaproteobacteria, including some known to oxidize Fe, were only observed in the estuarine samples and only present in very low abundance in the marine system.

• Corrosion experiments with mixed cultures of FeOB and FeRB grown in consortia. Used these to further establish overall surface effects of mixed cultures vs. pure cultures vs abiotic control. Profilometry replicates trends seen in previous experiments.

• Isolated two new stalk-forming FeOB: *Mariprofundus* strain DIS-1 from a corrosion coupon, and *Mariprofundus* strain GSB 1 from a local saltmarsh where corrosion studies were done. Both of these are obligate Fe-oxidizers. The genome of DIS-1 has been sequenced, and genomic evidence is consistent with physiological studies showing it is more tolerant of $O_2$ than other FeOB. Interestingly, DIS-1 is nearly identical by 16S rRNA gene sequence clones observed previously on steel coupons incubated in the open ocean, and is more distantly related the type strain *Mariprofundus ferrooxydans* PV-1, isolated from a hydrothermal vent. We are also in the process of sequencing the genome of GSB 1.

• A flow through reactor system was developed for studying biofilms that grow on steel coupons under controlled conditions in the laboratory. This system allowed control of $O_2$ levels, pH, flow rates, and supplemental iron additions, and was designed so steel coupons could be removed and viewed under a confocal microscope with minimal disruption. A novel technique was developed for imaging Fe-oxide rich biofilms using both lectins and DNA dyes confocal microscopy for simultaneous imaging of cells and biogenic iron oxides in 3-dimensions. This system was used to track growth of pure cultures of stalk-forming FeOB, originally isolated from natural enrichments, tracking growth, stalk-formation, and biofilm formation, and correlating this to environmental parameters. Data from these experiments, coupled with environmental data has led to a conceptual model that this mode of colonization may produce a complex surface area of cells and iron oxides that can be further colonized by other bacteria and may help mediate initiation of an MIC community.

• Applied single cell genomics sorting and amplification technology to corrosion samples. Isolated and amplified single cells (SAGs) with great success using this technique. Established better diversity estimates of Zetaproteobacteria observed in corroding biofilms, and are currently using these SAGs to understand more about metabolism of FeOB in general. We also obtained SAGs from a group of Epsilonproteobacteria there were numerically dominant in the corrosion communities.

• Used comparative genomics to identify potential genes involved in Fe-oxidation, and compared these to proteomic profiles of *Mariprofundus ferrooxydans* being grown on Fe(II) to identify key proteins involved in iron oxidation. This provide the first evidence for protein involved in neutrophilic Fe-oxidation.

• In addition to work related to the specific objectives for this project, we also conducted a study that investigated salinity effects on populations of marine and freshwater FeOB, showing that Zetaproteobacteria are not found in freshwater Fe seeps, but are more prevalent than freshwater FeOB even at very low salinities, suggesting they tolerate a wider salinity range than freshwater FeOB. We also contributed to a study of Fe-cycling in a freshwater Fe-seep, which demonstrated for the first time, active Fe-cycling within a discrete Fe-oxidizing microbial mat community.

**CONCLUSIONS** : The work done for this project has shown unequivocally shown that lithotrophic FeOB are capable of colonizing and growing on steel, using Fe(II) released from the steel surface as an energy source. It has shown there is hitherto unrecognized source of FeOB in coastal and estuarine environments. It has shown that FeOB are early colonizers of steel surfaces, and that while they are abundant during the early phase of colonization, their diversity is low, and there appears to be a successional progression to the corrosion community with time as other microbial populations, including anaerobes and putative sulfate-reducing bacteria, become more prevalent. Microcosm studies suggest FeOB cause roughening of steel surfaces and that there may be a synergistic coupling with Fe-reducing bacteria that further promotes degradation of the steel surface; however evidence for pitting and dramatic acceleration of corrosion by FeOB was not found. A microscopic method was developed to follow colonization and

growth of FeOB on steel coupons and found that they produce a loosely adherent, but thick (100's of micrometers) biofilm as a result of initial attachment to the steel surface and growth coupled with stalk formation. A model is being proposed that this mode of colonization may produce a complex surface area of cells and iron oxides that can be further colonized by other bacteria and may help mediate initiation of an MIC community. This work also led to the isolation of new strains of FeOB, studying these strains has led to new understandings about how bacteria oxidize iron.

**SIGNIFICANCE :** Our studies have provided novel information about an important member of the microbial corrosion community. Ultimately this work lays the groundwork for better methods to detect microbially influenced corrosion, understand the overall process of MIC, will ultimately be of use in develop means to mitigate this process and reduce costs and hazards related to biocorrosion.

**PATENT INFORMATION** : No patents applied for.

**AWARD INFORMATION** : PI has been awarded two grants from the National Science Foundation (total budgets approximately $1.1 million) for work on role of Fe-oxidizing bacteria at hydrothermal vents, and impacts on coastal iron cycling processes. Work from this project contributed in various ways to the success of these awards.

**PUBLICATIONS and ABSTRACTS** (for total period of grant):

**Proposals in preparation directly supported through this work.**

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McBeth, J.M., and D. Succession of Corrosion communities on mild Steel, and the role of Zetaproteobacteria. Frontiers in Microbiology – special issue honoring Katrina Edwards. In Prep.

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